# DECLARATION OF ROLAND KONTERMANN UNDER 37 C.F.R. § 1.132

#20 3/24/02

I, Roland Kontermann, do declare as follows:

1. I received a doctorate degree from the University of Heidelberg, Germany. I have worked in the field of protein engineering from 1992 until today.

2. I was a research associate at the MRC centre for Protein Engineering in Cambridge, UK from 1993 to 1996.

3. I have been working in the field of targeting technologies for drug delivery for different diseases (such as, e.g. cancer) since 1993. I have published 14 papers, presented 20 abstracts and held 13 oral presentations at scientific meetings in the field of protein engineering. I published 5 papers and 18 meeting abstracts and oral presentations on engineered proteins for tumour therapy. My curriculum vitae is attached as EXHIBIT A.

4. I have reviewed U.S. Patent application No. 09/256,237 ("the application") which describes a nucleic acid construct encoding a polypeptide, that comprises one or more active compounds and one or more inhibitory compounds connected to each other via one or more cleavable amino acid sequences. The construct described above allows for the therapy of different diseases such as cancer or inflammations by providing an inactive precursor of an active compound that is specifically activated at the target site (the tumour, inflammation), thus using traits characteristic for the target cells to allow for a local release of the active compound avoiding systemic side effects. One specific example given in the application refers to a nucleic acid construct encoding a PSA-activable factor X (FX).

5. I believe that as of the filing date of the application, (1997), the methods described in the application would have been believable to me or someone else skilled in the art of the invention. The feasibility of this approach was first shown when Watt et al. (Proc. Natl. Acad. Sci. USA, 83, 3166 – 3170, 1986) demonstrated that besides normal prostatic glandular cells, the only other cell type in males that secretes substantial amounts of PSA are prostate cancer cells. Physiological FX is a glycoprotein of the blood coagulation cascade that, together with factor Va, calcium and phospholipids, converts prothrombin to thrombin, thus leading to blood coagulation (Furie, B and Furie BC, Cell 1988; 53: 505-518) (EXHIBIT B). Conversion of FX into active FXa is caused by either factor IXa of the intrinsic coagulation pathway or by factor VIIa of the extrinsic pathway. This activation involves specific cleavage of an argininyl-isoleucyl peptide bond between the activation peptide and the catalytic domain.

By amino acid-exchanges in the activation cleavage site of FX the inventors of the U.S. Patent application No. 09/256,237 (in the following referred to as "the inventors") created a mutated factor X, unable to be cleaved by its predecessor in the coagulation cascade (the coagulation factors are very specific for their target sequences, thus safeguarding, that in the human body no coagulation takes place at random; compare Furie, B and Furie BC, Cell 1988; 53: 505-518 ) but able to be cleaved by PSA: The cleavability of the mutated factor X by PSA and its ability to activate the coagulation cascade in FX-deficient plasma was shown by the inventors by means of in vitro recalcification experiments: The recalcification assay is a standard in vitro system for measuring the coagulation activity. In this assay, the coagulation activity is determined by means of the time that passes until coagulation takes place in vitro (referred to as the recalcification time). In the inventor's experiments, when mutated factor X, together with PSA is added to the factor X-deficient plasma, the recalcification time is drastically reduced in comparison to the negative control, indicating that the mutated factor X can be cleaved by PSA and is

able to initiate blood coagulation in vitro. Moreover, the data demonstrate, that the combination of mutated factor X and PSA is as effective in inducing blood coagulation in vitro as wild-type factor X, when cleaved by Russel's Viper Venom (a standard positive control for the induction of blood coagulation by factor X). Since the assay basically contains all components necessary for blood coagulation in vivo (with exception of Calcium, which is used as a starter, and factor X), among persons of skill in the art of the invention the assay is commonly accepted as a credible model of the blood coagulation in the body, its results being predictive of the situation in vivo. Thus, it can be expected that mutated factor X is cleaved and activated by PSA and induces blood coagulation in vivo, too.

6. As of the date of the invention, therapies based on cell targeting had gained acceptance in the art. The disadvantage of classical therapeutic approaches against cancer is the ability of cancerous cells to develop resistances against the drugs employed. thus evading most therapeutic approaches. Earlier studies had already shown that a functional tumour vasculature is essential for the tumours growth, invasivity and potential to metastasise (Liotta et al, Cancer Res. 34: 997-1004, Folkman et al., 1989 Nature 339: 58-61). (EXHIBITS C and D, respectively) Thus, Folkman et. al developed an anti-angiogenic approach targeting the genetically stable tumour-endothelium rather than the cancerous cells, since endothelial cells do not develop resistances as readily as the heterogeneous, genetically instable tumour cells do (Folkman et al. 1994, N. Engl. J. Med. 333, (26) 1757-1763; Folkman J. 1995, Nature Medicine 1: 27-31). (EXHIBITS E and F, respectively) This therapeutic approach is expected to be very efficient since the partial destruction of the tumour endothelium leads to an intravasal coagulation resulting in a vessel obstruction that stops the tumours blood supply. The feasibility of a vasculature-directed approach was later-on also demonstrated by Huang et al. (Science 1997, 275: 547-50) (EXHIBIT G) showing that activation of blood coagulation in tumours by specific targeting of tissue factor to tumour endothelium causes necrosis and remission of the

tumour. A further advantage of the above-indicated approaches is that the difference of the underlying principle to classical chemo therapies allows for the synergistic combination of this therapy with classical approaches.

Based on these findings, it should be possible to induce blood coagulation and tumour necrosis using coagulation factor X variants with modified activation cleavage sites, so they can be activated by tumour-associated proteases such as PSA. The specific secretion of PSA by prostatic glandular cells and prostate cancer cells, together with its ready inactivation in the blood stream by plasma protease inhibitors such as a-2 macroglobulin (Leinonen et al. 1996, J. Urol. 155: 1099-1103) (EXHIBIT H) and a-1-antichymotrypsin (Lilja et al. 1991, Clin. Chem. 37: 1618-1625)(EXHIBIT I) render its target amino acid sequence an excellent candidate for the cleavable amino acid sequence according to the invention. (In fact, experiments by Denmeade et al. using PSA in order to activate a doxorubicin-peptide prodrug, later on gave proof of the excellent usability of PSA for a therapy according to the invention in vitro (Denmeade et al., 1997 Cancer Res. 57, 4924-30 and especially Denmeade et al. 1998, Cancer Res. 58, 2537-40). Even more recent findings of DeFeo-Jones et al. (DeFeo-Jones et al. 2000, Nature Medicine Vol..6, No.11: 1248-1252) (EXHIBIT J) demonstrated the ability of PSA to activate a peptide-doxorubicin 'prodrug' in the animal model, leading to selective killing of prostate tumour cells in vivo.)

7. In the light of all the above findings, a polypeptide according to the invention of the application comprising the mutated FX, after systemic (e.g. intravenous or intraperitoneal) application would be distributed by the blood stream and transported to the target site in an inactive (inhibited) state. Once in the tumour vasculature, it would meet active PSA molecules in the immediate vicinity of the PSA-secreting tumour cells (in the blood vessels or in the interstitial space between tumour tissue and blood vessels) able to cleave the cleavable amino acid sequence comprising the PSA target sequence, and setting free the active mutated FX, thus

initiating the blood coagulation cascade and consequentially leading to a stop of the tumours blood supply, which then would either impair its growth and potential to invade the surrounding tissue and to metastasise or result in a complete remission of the tumour.

8. The general applicability of the invention was credible as of the filing date of the application: The above example of the mutated factor X readily demonstrates that the activity of an active compound can be targeted to a specific target cell or target tissue by modifying the activation cleavage site of its inactive precursor in such a way that it can be cleaved exclusively by a protease released by the target cell itself or by a protease released at (i.e. in the vicinity of) the target cell. The active compound can be any compound exerting a negative effect (such as, e.g. factor X-initiated coagulation resulting in the necrosis of the target tissue) or a positive effect (e.g. neurotrophic growth factors, such as NGF, BDNF or the like, relieving damage to the nervous system) on the target cell or the target tissue. Thus, target cells of solid tissues (e.g. tumour cells of solid tumours; neurons; inflammatory cells in arthritic cartilage or connective tissue cells in arthritic cartilage, etc.) can be targeted by incorporating activation cleavage sites, that can be specifically cleaved by proteases secreted by the target cells themselves or by proteases secreted by other cells specific for the target tissue (i.e. specific tumour-endothelial cells; glia cells; connective tissue cells, etc.), into inactive precursors of active compounds. Moreover, the invention can be used to target cells within the body fluids or cells that are not restricted to specific tissues (such as cells of the immune system, metastasising cells of solid tumours, lymphoma cells or the like), by chosing an activation cleavage site that is specifically cleaved by a protease that is exclusively expressed by the target cells.

9. I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true;

and further that these statements and the like are made with knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardise the validity of this application or any patent issuing thereon.

Date: March 6, 2002 Signature: R. Kuller